Cytogenetic Characterization of Selected Small Round Cell Tumors of Childhood*

J. Whang-Peng, T. J. Triche, T. Knutsen, J. Miser, S. Kao-Shan, S. Tsai, and M. A. Israel

ABSTRACT: *Small, round, blue-cell tumors (SRCT), including rhabdomyosarcoma, Ewing's sarcoma of bone and soft tissue, mesenchymal chondrosarcoma, small cell osteosarcoma, hemangiopericytoma, neuroblastoma, peripheral neurectodermal tumor (peripheral neuroepithelioma of bone and soft tissue), and the malignant small cell tumor of the thoracopulmonary region described by Askin (Askin's tumor), are often difficult to distinguish by light microscopy. We have evaluated the cytogenetics of these tumors by studying 24 tumor explants in short-term culture and 22 tumor cell lines. In Ewing's sarcoma (a tumor of unknown histogenesis), and in peripheral neuroepithelioma and Askin's tumor (tumors with evidence of neural origin), we have observed an indistinguishable t(11;22) translocation.*

INTRODUCTION

The development of chromosome banding techniques has resulted in significant progress in the cytogenetic characterization of solid tumors of children and young adults. One subset of these tumors, the small, round, blue-cell tumors (SRCT) (Table 1), which includes rhabdomyosarcoma, Ewing's sarcoma of bone and soft tissue, mesenchymal chondrosarcoma, small cell osteosarcoma, hemangiopericytoma, neuroblastoma, and peripheral neuroectodermal tumors (both peripheral neuroepithelioma of bone and soft tissue and the small cell tumor of the thoracopulmonary region described by Askin [1]), are often difficult to definitively distinguish by light microscopy alone. In an attempt to further compare and contrast these SRCT of childhood, we have evaluated the cytogenetics of these tumors by studying 24 tumor explants in short-term culture and 22 tumor cell lines.

*This article is part of the First International Workshop on Chromosomes in Solid Tumors (Tucson, AZ, March 3–5, 1985), which was published in *Cancer Genetics and Cytogenetics* 19(1&2), 1986.

From the National Cancer Institute, National Institutes of Health, Bethesda, Md (J. W-P., T. J. T., T. K., J. M., S. K-O., M. A. I.), and Biotech, Inc., Rockville, MD (S. T.).

Address requests for reprints to Dr. Jacqueline Whang-Peng, Chief, Cytogenetic Oncology Section, M. B., National Cancer Institute, Building 10, Rm. 12N226, National Institutes of Health, Bethesda, MD 20892.

Received August 5, 1985; accepted August 19, 1985.

Cancer Genet Cytogenet 21:185–208 (1986)
0165-4608/86/$03.30

Table 1 Classification of SRCT

Soft Tissue
Rhabdomyosarcoma
Soft tissue (extraosseous) Ewing's sarcoma
Hemangiopericytoma
Bone
Ewing's sarcoma
Small cell osteosarcoma
Mesenchymal chondrosarcoma
Hemangiopericytoma of bone
Neural
Neuroblastoma
Peripheral neuroctodermal tumors
Askin's tumor
Neuroepithelioma
Pheochromocytoma

MATERIALS AND METHODS

Direct and Short-Term Cultures

Direct cytogenetic studies were performed on bone marrow aspirates, pleural fluids, and tumor samples obtained from 24 patients referred to the Pediatric Oncology Branch, NCI, Clinical Center, NIH (see APPENDIX for brief case histories). There were eight cases of Ewing's sarcoma, three Askin's tumors of the chest wall, one soft tissue (extraosseous) Ewing's sarcoma, three peripheral neuroepitheliomas, two rhabdomyosarcomas, one ganglioneuroblastoma, and six pheochromocytomas. Tumor tissue was minced and then forced through an 190-micron pore size stainless steel mesh (Cellector, Bellco Glass, Vineland, NJ) to obtain single cell suspensions. Cells were collected either in a colcemid solution for direct preparation, or in RPMI 1640 medium (Grand Island Biological Company, Grand Island, NY) supplemented with 15% fetal calf serum and penicillin–streptomycin, and cultured for 1–4 days. After a short exposure to colcemid (1 hour at 0.2 μg/cc), the cells were treated with a hypotonic solution (1:1, 0.075M KCl/1% sodium citrate) for 20 minutes and then fixed in Carnoy's fixative (3:1, glacial acetic acid/absolute ethanol). The cells were then washed in several changes of fixative and slides were prepared by the air-dry method. The chromosome preparations were stained with conventional Giemsa and trypsin–Giemsa banding stains [2]. Fifty metaphases (when possible) were examined from each sample and karyotyped according to the Paris Conference standardization [3].

Establishment of Long-term Tumor Cell Lines

Biopsy specimens of solid tumors from the operating room were maintained under sterile conditions on wet gauze soaked in physiologic saline. As soon as possible, the tissue was finely minced, suspended in RPMI 1640 medium, and washed once by low speed centrifugation. Pelleted tissue was then resuspended in a minimal volume of medium containing 15% fetal calf serum and incubated at 37°C in 5% CO_2 until a cell line emerged or viability of the culture could no longer be maintained.

Long-term tissue culture lines were studied from 22 individuals (13 of the 22 were also studied by direct tumor sampling), including 13 Ewing's patients, five

Table 2 Ewing's sarcoma

PT/TC[a]	Age (yr)/Sex	Specimen[b]	Culture[c]	Modal number (range)	Clonal chromosomal abnormalities in ≥50% of cells[d]
3-13	13/M	T	dir	46(44–46)	t(11;22)(q23.3;q12)
2-16	16/M	BM	6 days	46(43–46)	t(11;22)(q24;q12)
17-11	4/M	T	dir	No mitoses	
2-19	10/M	T	dir	No mitoses	
19-4	23/M	T	dir	No mitoses	
10-20	16/M	T	dir	51 (51)	+5,+8,+12,+15,+18,t(11;22)(q21;q12)
10-20/NX1024		T	12 days	51(49–54)	+5,+8,+12,+15,+18,t(2;4)(q37;q13),t(11;22)(q21;q12)
10-2	28/M	BM	dir	46(46–49)	+8,t(6;12)(p13;q14),t(11;22)(q24;q12),del(12)(q14)
10-2/TC128		T	2 mo	46(44–47)	+8,t(6;12)(p13;q14),t(11;22)(q24;q12),del(12)(q14)
5-23	5/M	T	dir	No mitoses	
5-23/TC180		T	3 wk	47(45–47)	−7,+12,+18,t(10;21)(q12;p11),t(11;18;22)(q24;q22;q12)
TC71	22/M	T recur	1981	76,80(65–84)	−Y,−8,t(1;7)(q25;p11),del(2)(q36),t(2;14)(q12;q32)3q+,?5,del(6)(q26), del(7)(q31),t(7;11)(q21;q23),t(8;14)(q11;p11),t(11;22)(q24;q12)
TC106	19/M	T	1982	49(47–52)	+5,+15,−18,t(5;17)(q12;q22),del(8)(p22),del(10)(q31),t(11;17)(q24;q22), t(11;22)(q24;q12)
A4573	17/F	PF	1976	71(52–78)	t(1;16)(q22;q24),t(2;6)(q34;q27),t(2;9)(q13;p24),t(11;22)(q24;q12), i(15q),20q+
#5838	27/M	PF	1974	77(49–92)	+X,+Y,del(2)(p12),del(6)(q24),9p+,t(11;22)(q24;q12),2-4 small acro
#6647	27/F	PF	1974	47(43–53)	+20,del(X)(q23),i(1q),t(11;22)(q24;q12),22q+
A9423	13/F	LN	1977	44(42–90)	−5,−19,del(X)(q26),del(1)(p31),1p+,1p+,del(2)(q32),acentric fragment(?4q),t(11;22)(q24;q12)
N1001	11/F	BM	1983	50(38–57)	−12,−17,+20,+21,del(X)(q25),del(X)(q27),t(1;16)(q24;q23),t(9;14)(p13;q11), t(11;22;17)(q24;q12;q22)
N1002	25/M	BM	1983	58,59(51–63)	+Y,+4,+5,+6,+7,+8,+9,+13,+20,+21,del(1)(p21),t(11;22)(q24;q12), t(14;15)(q11;p11)
N1003	18/M	BM	1983	45,46(43–58)	i dup(1)(qter→q22::qter→cen→qter),t(1;6)(q22;q27),t(10;14)(p11;q11), del(13)(q13q14),t(11;22)(q24;q12),min
N1007	19/M	BM	1984	48,49(44–50)	−Y,del(X)(q25),t(X;7)(q25;q36),del(5)(:p14→q33:),t(11;22)(q24;q12)

[a]PT/TC, patient/tissue culture ID.

[b]T, tumor; BM, bone marrow; PF, pleural fluid; LN, lymph node; recur, recurrence.

[c]Culture refers to direct harvest or short-term culture of tissue specimens or date of establishment of tumor cell lines; dir, direct or 1–4 culture harvest.

[d]Underscoring indicates translocations involving #11 and #22.

Askin's, one soft-tissue Ewing's, two peripheral neuroepithelioma, and one rhabdomyosarcoma. The oldest cell line was started in 1974. Some of the older lines were derived by G. Cannon (Litton Bionetics Corporation, Rockville, MD). See APPENDIX for case histories.

RESULTS

Tables 2, 3, and 4 contain the following data for each specimen: the age and sex of the patient, specimen type, chromosome number (modal and range), and clonal abnormalities observed in 50% or more of all metaphases examined. A summary of the occurrence of a t(11;22) marker in the various diseases is shown in Table 5 and the translocation is divided into three categories: typical translocation [t(11;22)(q24;q12)], complex translocation (involving three or more chromosomes), and presence of other translocations without t(11;22).

Ewing's Sarcoma

Direct cytogenetic studies of tumor or tumor-involved bone marrow were made for eight patients; successful preparations were obtained in four patients. Two patients (3-13, 2-6) had pseudodiploid karyotypes with the previously described t(11;22)(q24;q12) translocation [4] (Fig. 1). A third patient (10-20) had a hyperdiploid karyotype with t(11;22) showing a higher breakpoint on chromosome #11, t(11;22)(q21;q12) by direct study, whereas, a tissue culture line (N1024) from the latter patient showed the same translocaton, numerical abnormalities, and t(2;4)(q37;q13) (Fig. 2). A fourth patient (10-2) had t(11;22)(q24;q12) in both the direct specimen and the cell line (TC128) derived from his tumor. A translocation involving chromosomes #6 and #12, t(6;12)(p13;q14), was also observed in the specimens from this patient (Figure 3A).

In another case (5-23) where both direct tumor sampling and tissue culture studies were performed, no mitoses were obtained in the direct sample, but karyotypes obtained from the established cell line (TC180) showed a complex translocation, t(11;18;22)(q24;q22;q12), and −7, +12, +18, and t(10;21)(q12;p11).

Tissue culture cell lines established from a variety of tissues were available from ten additional patients with Ewing's sarcoma referred to the NCI: two were initiated directly from tumor tissue, three from pleural fluid, one from lymph node, and four from bone marrow. All of them had a translocation between chromosomes #11 and

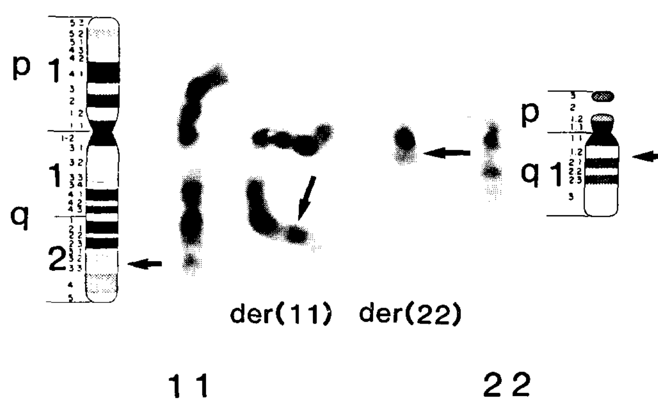


Figure 1 Partial karyotype from a direct tumor preparation from patient 3-13 (Ewing's sarcoma) showing t(11;22)(q23.3; q11.2). Arrows indicate location of breakpoints in the patient's chromosomes and the idiograms of chromosomes #11 and #22.

Table 3 Askin's tumor and peripheral neuroepithelioma

PT/TC[a]	Age (yr)/Sex	Specimen[b]	Culture[c]	Modal number (range)	Clonal chromosomal abnormalities in ≥50% of cells[d]
ASKIN'S TUMOR					
19-19	22/M	BM	dir	46[e],48(42–48)	+11,+18,t(11;22)(q24;q12)
19-19/NX1012		BM	2 wk	46(41–47)	Normal (bone marrow)
19-7	16/F	BM	dir	47,48(46–49)	t(11;22)(q24;q12)
19-7/N1016		BM	9 days	46(42–47)	−X,+7,t(11;22)(q24;q12)
20-8	33/F	PF	dir	No mitoses	
20-8/N1008		PF	2 mo	48(45–51)	+8,+16,22q12−
SKNMC		T	1973	46(43–48)	+8,−10,t(1;3)(q22;q27),t(1;4)(p36;p13),t(2;11;22;21)(q32;q24;q12;p11)
CHP100		T	1972	46,47;90,92(42–94)	t(1q;9q;11q),del(3)(p14),del(3)(q21),t(4;22)(q13;q13),t(7;16)(q21;q24), t(11;22)(q24;q12)
PERIPHERAL NEUROEPITHELIOMA					
10-15	5/M	T	dir	No mitoses	
11-15	17/F	BM	dir	46	t(11;22)(q24;q12)
11-15/TC32		T	17 mo	48(46–49)	+5,+10,i(1q),t(11;22)(q24;q12)
20-3	14/M	T	dir	46,47;51-53(46–53)	+4,+5,+6,+12,+22,t(11;22)(q24;q12)
20-3/N1000		T	2 mo	47(43–53)	+4,+5,+6,+8,+12,+18,+22,t(11;22)(q24;q12)

[a,b,c,d]See Table 2 footnote.

[e]The cells with 46 chromosomes had a normal karyotype.

Table 4 Soft tissue (extraosseous) Ewing's sarcoma, rhabdomyosarcoma, and ganglioneuroblastoma

PT/TC[a]	Age (yr)/Sex	Specimen[b]	Culture[c]	Modal number (range)	Clonal chromosomal abnormalities in ≥50% of cells
SOFT TISSUE (EXTRAOSSEOUS) EWING'S SARCOMA					
13-2	25/M	T	dir	46(45–46)	t(6;12)(q14;p13)
13-2/NM1010		T	2 mo	46(42–47)	t(6;12)(q14;p13)
RHABDOMYOSARCOMA					
4-2	14/F	BM	dir	46,84–88(46–92)	t(2;11)(q27;q13),i(10q),14q+
4-8	10/F	T	dir	No mitoses	
TC-131T	19/M	T	10 mo	54(48–61)	+X,+1,+3,+8,+11,+12,+20,del(6)(q22),t(4;13)(q13;q34)
GANGLIONEUROBLASTOMA					
11-7	21/M	PF	3 days	46(45–46)	Numerical change, 4 cells: Chromosome #1/4 cells (p arm, 1;q arm, 3) Structural change, 7 cells: Chromosome #11/3 cells (p arm, 3;q arm, 1) Chromosome #12/3 cells (q12)
		PB	3 days	46(43–49)	Numerical change, 1 cell: +16 Structural change, 4 cells: t(1;19),t(7;22),t(2;5),t(7;15),t(4;8),t(11;17),t(4;17)

[a],[b],[c]See Table 2 footnote.

Table 5 Chromosome translocations in SRCT

Disease	Direct: Number of patients Succ (Total)	Direct: t(11;22) t(11;22)(q24;q12)	Direct: t(11;22) Complex or diff	Direct: No t(11;22) but other translocations	Culture: Number of patients Succ (Total)	Culture: t(11;22) t(11;22)(q24;q12)	Culture: t(11;22) Complex or diff	Culture: No t(11;22) but other translocations
Soft tissue								
Rhabdomyosarcoma	1 (2)			1	1 (1)			1
Extraosseous (soft tissue) Ewing's sarcoma	1 (1)			1[a]	1 (1)			1[a]
Bone tumor								
Ewing's Sarcoma	4 (8)	3[b]	1 t(11;22)(q21;q12)		13 (13)	10[b]	3 t(11;22)(q21;q12) t(11;18;22)(q24;q22;q12) t(11;22;17)(q24;q12;q22)	
Neural tumor								
Peripheral Neuroepithelioma	2 (3)	2			2 (2)	2		
Askin's Tumor	2 (3)	2			5 (5)[c]	2	1 t(2;11;22;21) (q32;q24;q12;p11)	1[c]
Pheochromocytoma	0 (6)							
Ganglioneuroblastoma	1 (1)			1				
Total	11 (24)				22 (22)			

[a] t(6;12)(qter→q14;p13)

[b] One patient also had t(6;12)(p13;q14).

[c] One patient had a normal karyotype.

[d] One patient had only del(22)(q12) with no translocation involving #11.

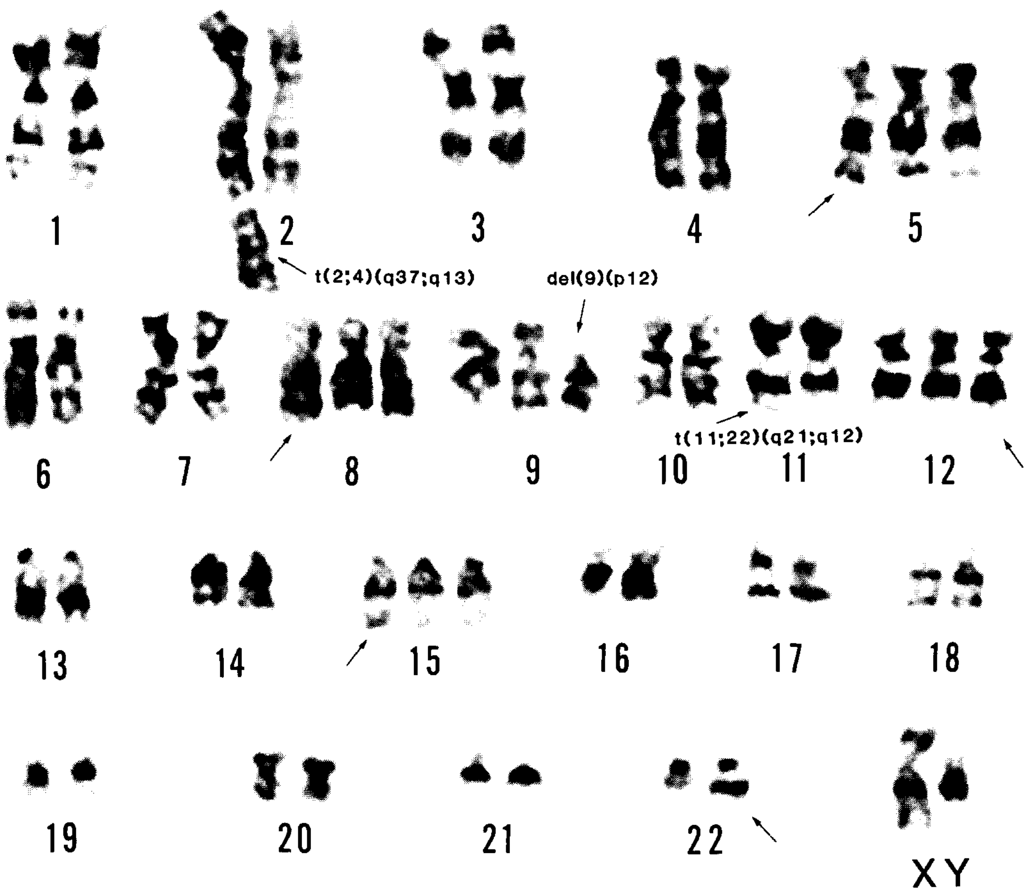


Figure 2 Karyotype from tumor cell lines N1024, established from a male patient with Ewing's sarcoma: 51,XY,+5,+8,+12,+15,t(2;4)(q37;q13),del(9)(p12),t(11;22)(q21;q12).

#22: nine cultures had t(11;22)(q24;q21) (Figs. 4 and 5) and one had a complex translocation, t(11;22;17)(q24;q12;q22) (Fig. 6).

Askin's Tumor

Cytogenetic studies were performed on tumor tissue and on cell lines established from tumor tissue obtained at biopsy from three patients with Askin's tumor. Preparations adequate for study were obtained from tumor tissue from two patients (19-19 and 19-7). These studies revealed chromosome numbers in the diploid range and t(11;22) (Fig. 7). In the first patient (19-19) a cell line derived from tumor aspirated from the bone marrow (NX1012) showed a normal karyotype and in the other patient (19-7) a tumor cell line (N1016) showed t(11;22) (Fig. 7). In the third patient (20-8) direct studies were unsuccessful and the tissue culture (N1008) had 45–51 chromosomes and del(22)(q12) with no clear evidence of a translocation site. Two additional cell lines (SKNMC and CHP100) that were studied had numerous chromosomal abnormalities and chromosome numbers in the diploid (SKNMC) or diploid/tetraploid (CHP100) ranges. Tissue culture CHP100 had t(11;22)(q24;q12) and SKNMC had a complex translocation, t(2;11;22;21) (Fig. 7).

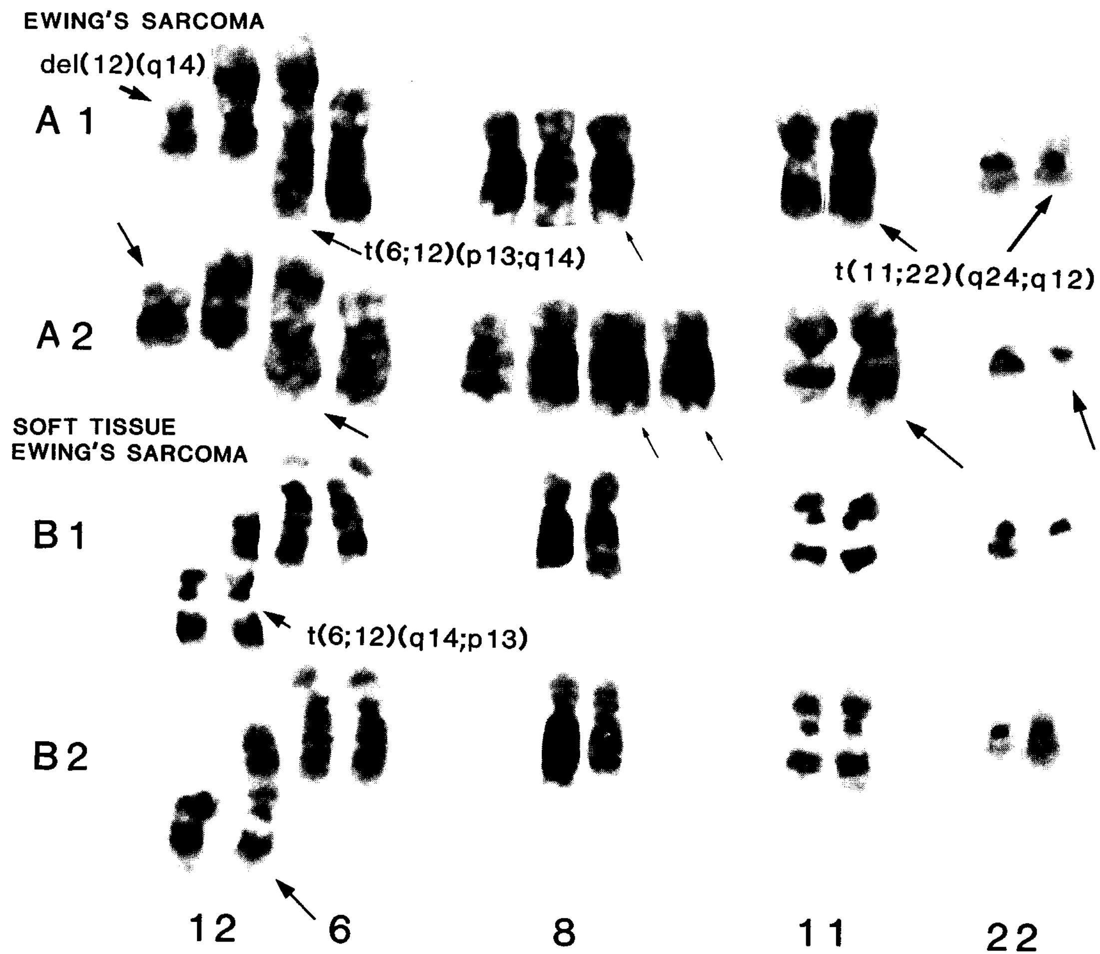


Figure 3 Partial karyotypes showing t(6;12) in two patients. *Patient 10-2* (Ewing's sarcoma): (A1) +8,t(6;12)(p13;q14),t(11;22)(q24;q12); (A2) +8,+8,t(6;12)(p13;q14),t(11;22)(q24;q12). *Patient 13-2* (soft tissue Ewing's sarcoma): (B1) t(6;12)(q14;p13); (B2) t(6;12)(q14;p13).

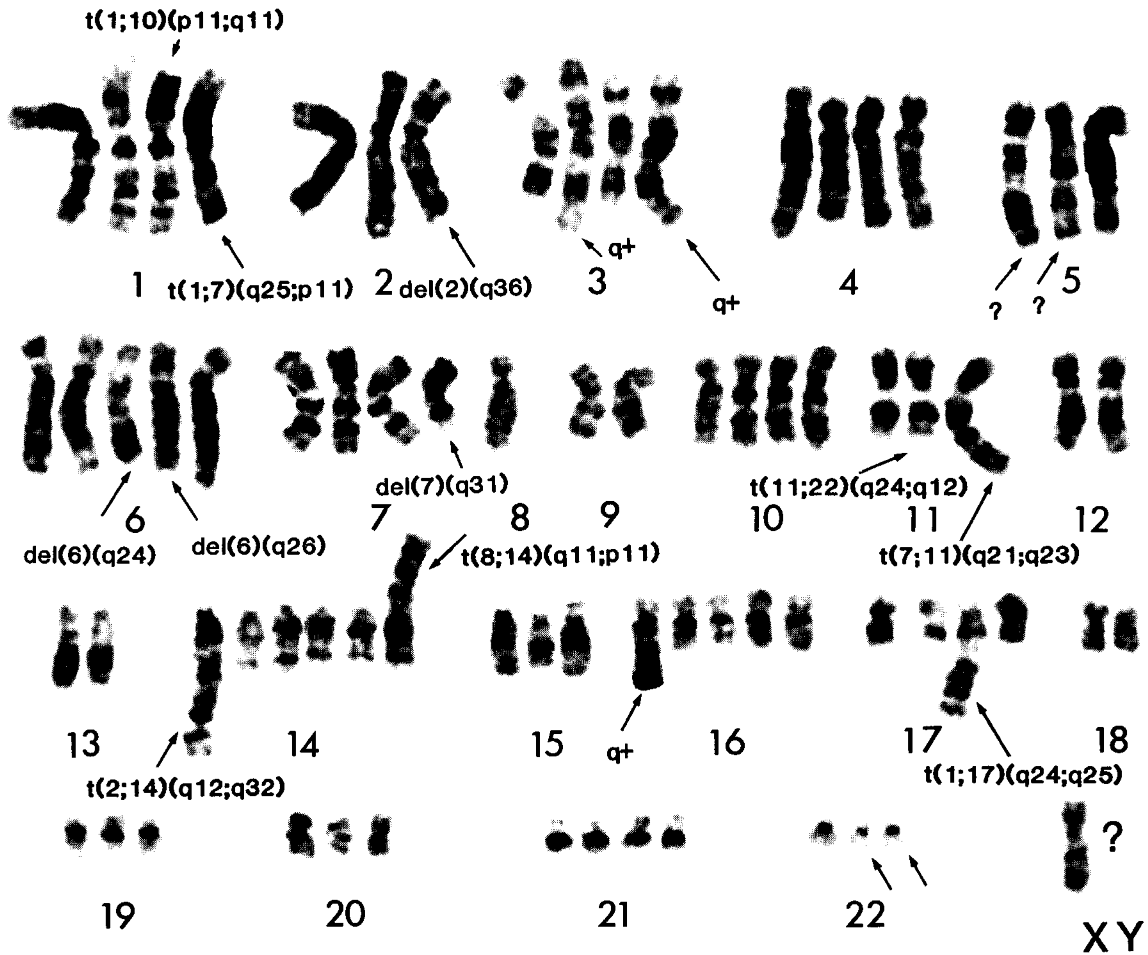


Figure 4 Karyotype from tumor cell line TC71, established from a male patient with Ewing's sarcoma, showing 75 chromosomes and multiple numerical and structural abnormalities including two different translocations involving chromosome #11: t(11;22)(q24;q12) and t(7;11)(q21;q23).

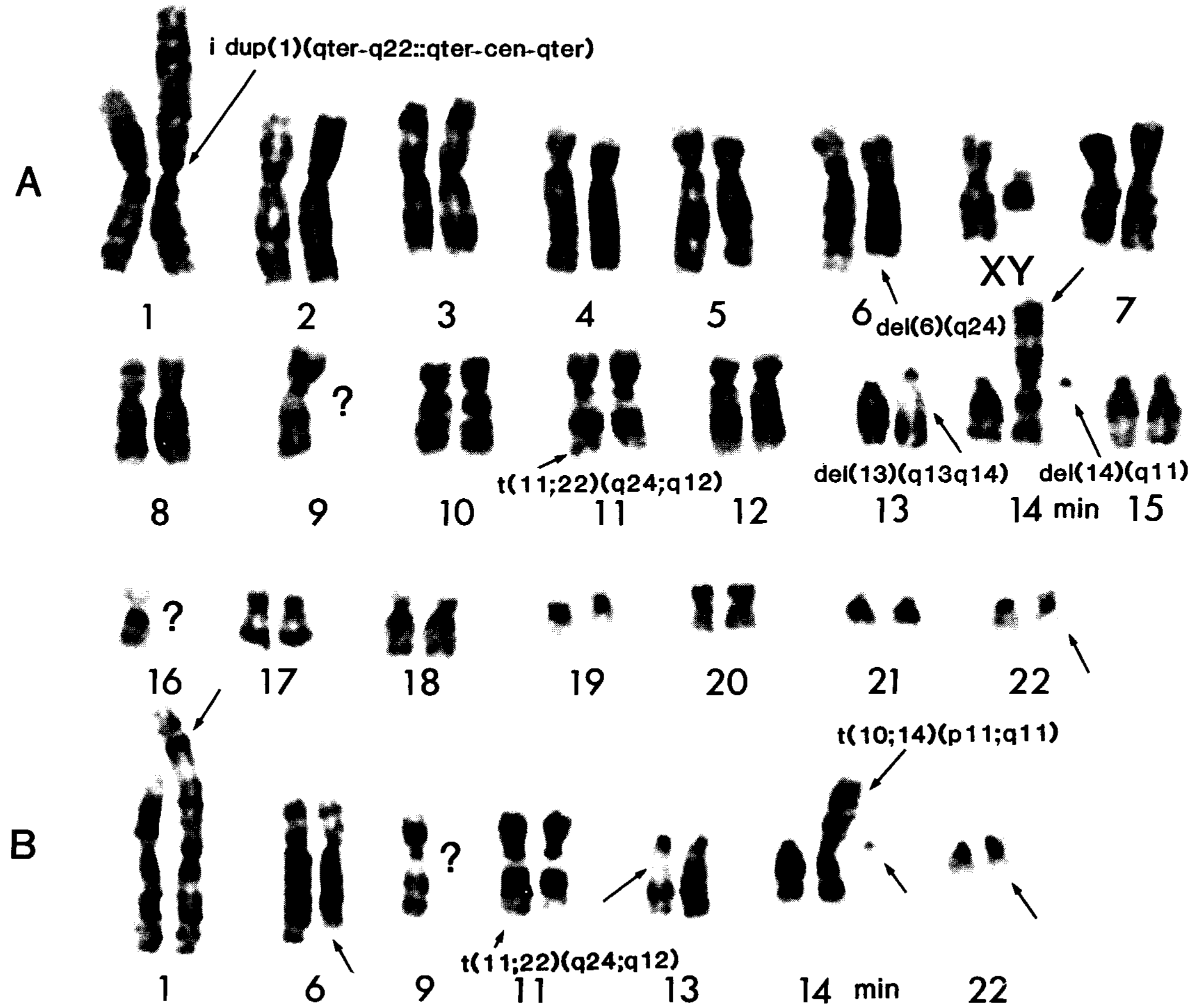


Figure 5 Two karyotypes from bone marrow cell line N1003, established from a male patient with Ewing's sarcoma. i dup(1)(qter→q22::qter→cen→qter),del(6)(q24) (A) 45XY, −9, −16,t(10;14)(p11;q11),t(11;22)(q24;q12),del(13)(q13q14), +min[del(14)(q11)]. (B) Partial karyotype showing the same structural abnormalities as in (A).

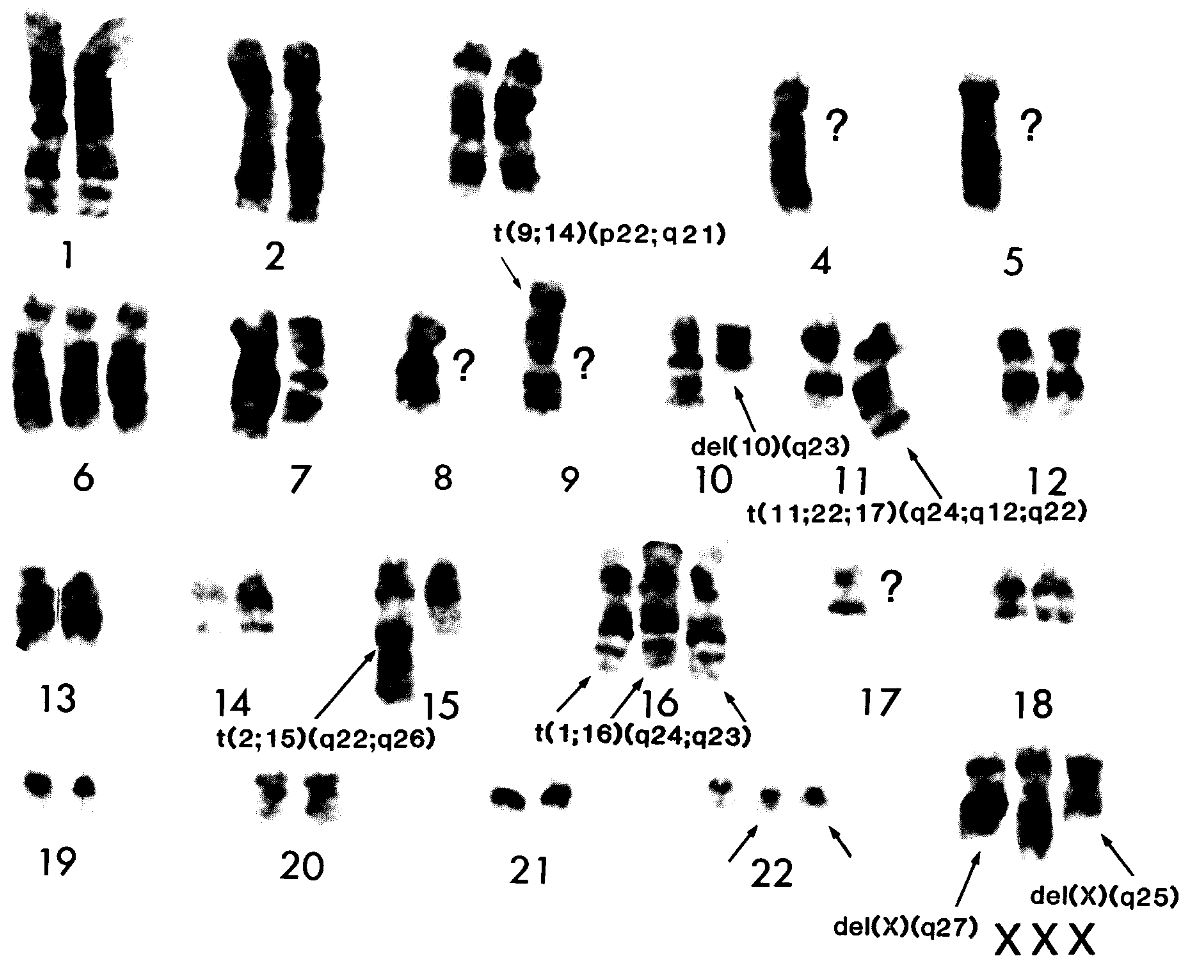


Figure 6 Karyotype from bone marrow cell line N1001, established from a female patient with Ewing's sarcoma: 45,X, del(X)(q27),del(X)(q25), −4, −5, +6, −8, −9, −17,t(1;16)(q24;q23),t(1;16)(q24;q23), + t(1;16)(q24;q23),t(2;15)(q22;q26), t(9;14)(p22;q21),del(10)(q23),t(11;22;17)(q24;q12;q22).

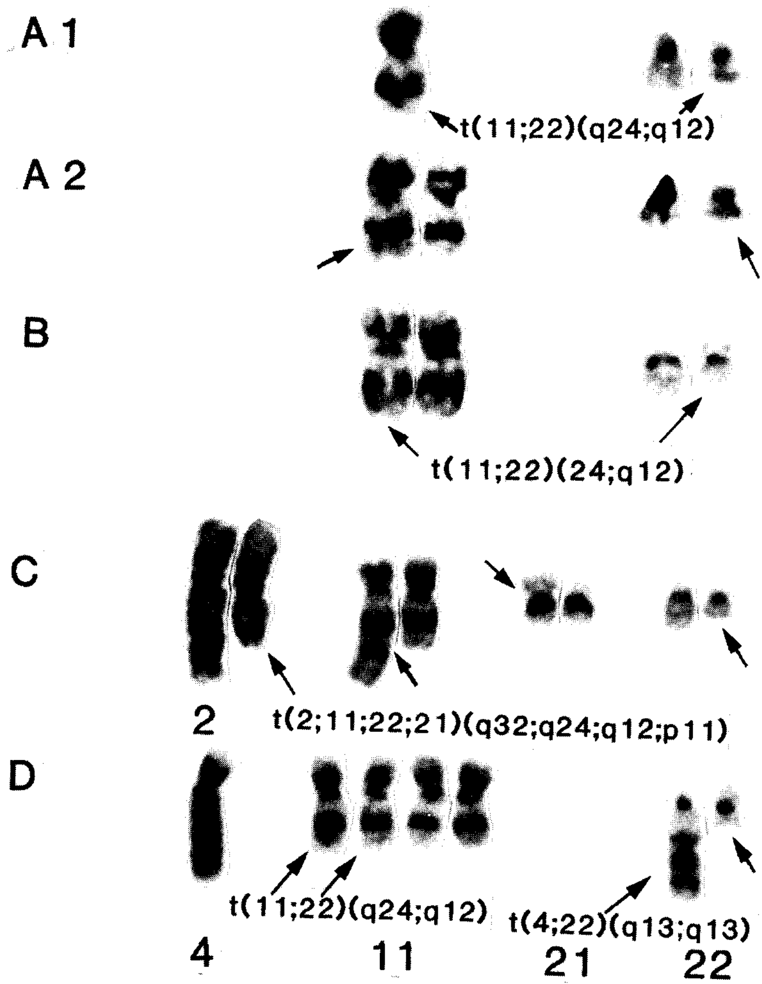


Figure 7 Partial karyotypes from four patients with Askin's tumor. (A1) *Patient 19-7* (bone marrow): −11,t(11;22)(q24;q12); (A2) cell line N1016, established from patient 19-7: t(11;22)(q24;q12); (B) cell line N1008, established from patient 20-8 (pleural fluid): t(11;22)(q24;q12); (C) cell line SKNMC (tumor): t(2;11;22;21)(q32;q24;q12;p11); (D) cell line CHP100 (tumor): t(11;22)(q24;q12) and t(4;22)(q13;q13).

Peripheral Neuroepithelioma

Direct studies were performed on three patients (10-15, 11-15, and 20-3; 11-15 and 20-3 have been previously reported) [5]. No mitoses were observed in tumor preparations from one patient (10-15). Tissue culture lines were established from the two patients (TC32 from 11-15, and N1000 from 20-3). A t(11;22)(q24;q12) translocation was observed in both the direct preparations and the cell lines from these two patients (Fig. 8).

Soft Tissue Ewing's Sarcoma

Cytogenetic analyses of both tumor tissue and a tumor cell line were performed on one patient (13-2). No t(11;22) was found, but a t(6;12)(q14;p13) was observed in both specimens (Fig. 3B).

Figure 8 Partial karyotypes from two patients with peripheral neuroepithelioma. (A1) *Patient 11-15* (bone marrow): t(11;22)(q24;q12); (A2) Cell line TC32, established from tumor from patient 11-15: i(1q), + 8,t(11;22)(q24;q12) (B) *Patient 20-3* (tumor): + 4, + 6, + 8, + 12, + 18, + 22,t(11;22)(q24;q12).

Rhabdomyosarcoma

Cytogenetic analyses of tumor specimens from two patients (4-2 and 4-8) and a tumor cell line from a third patient (TC-131T) were attempted. Although no mitoses were found in direct examination of the tumor from one patient (4-8), the tumor specimen from the other patient (4-2) had karyotypically normal diploid cells and hypotetraploid cells (84–88 chromosomes) containing t(2;11)(q27;q13),i(10q), and 14q+ (Fig. 9). The tissue culture line TC-131T had a modal number of 54 chromosomes and most cells contained +X,+1,+3,+8,+8,+11,+12,+20, +20,del(6)(q22), and t(4;13)(q13;q34) (Fig. 10). Neither of these samples showed any evidence of a translocation between chromosomes #11 and #22, nor a deletion of 22q.

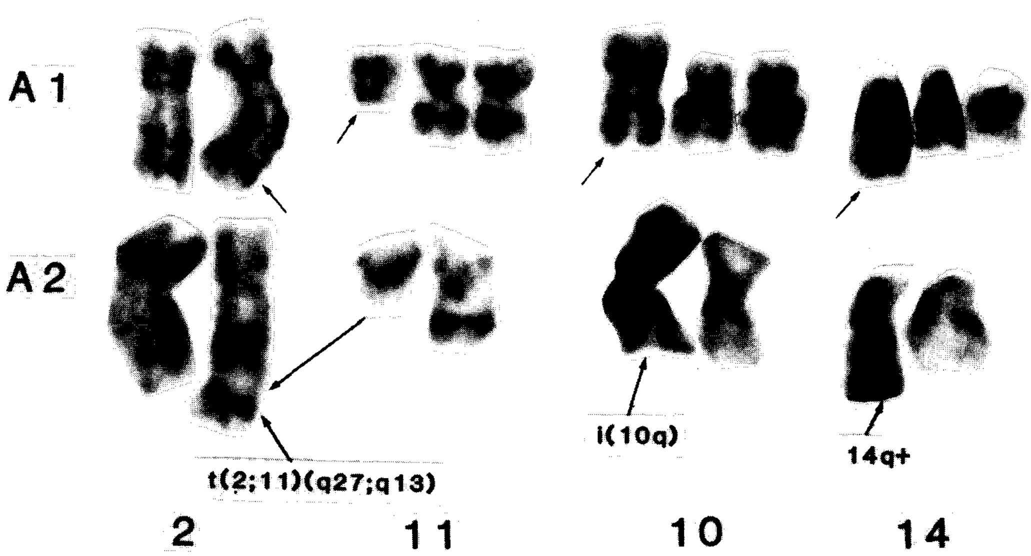
Ganglioneuroblastoma

Chromosome preparations were made from short-term culture of pleural fluid and peripheral blood from the only patient studied (11-7). Two-thirds of the mitoses examined in the pleural fluid showed structural and numerical abnormalities. Although no clonal abnormalities were observed, the structural abnormalities involved chromosomes #11 and #12. One-third of the peripheral blood metaphases also showed structural and numerical abnormalities that involved 11 different chromosomes but, again, without evidence of a clonal rearrangement (Fig. 11).

Pheochromocytoma

Cytogenetic analysis was performed on tumor tissue from six patients; however, no mitoses were observed in any of the samples.

Figure 9 Two partial karyotypes from direct bone marrow from rhabdomyosarcoma patient 4-2: t(2;11)(q27;q13),i(10q),14q+.



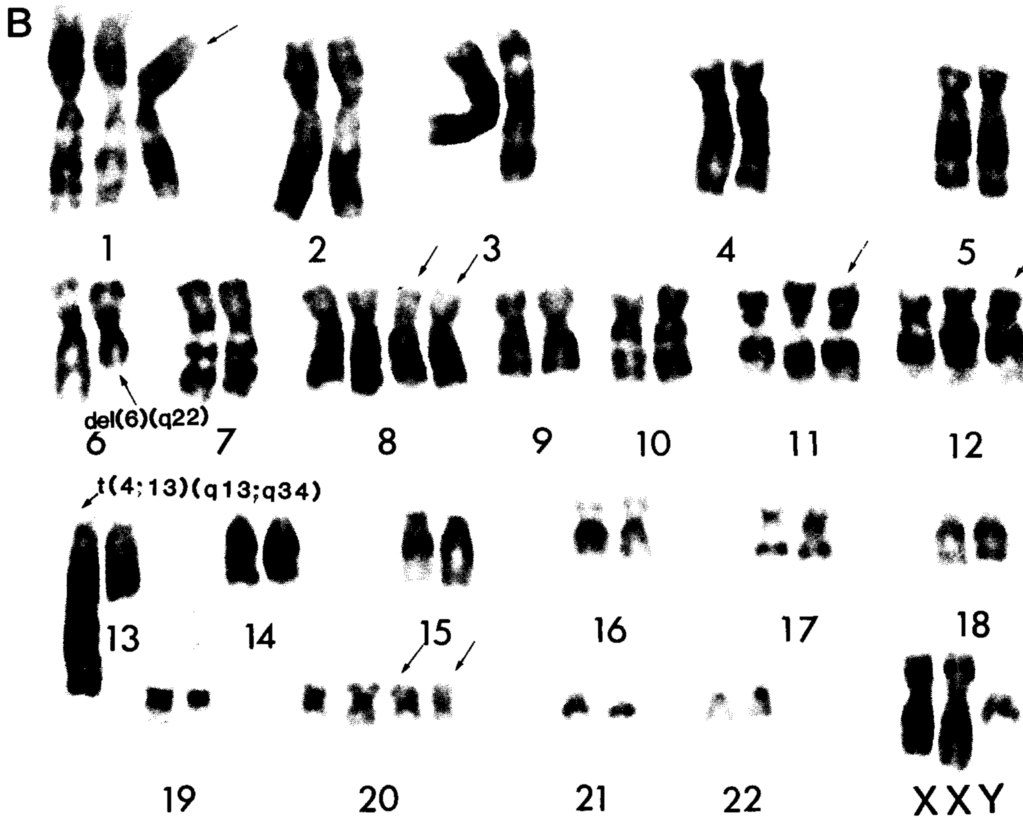


Figure 10 Karyotype from tumor cell line TC-131T, established from a patient with rhabdomyosarcoma: 54,XXY,+1,+8,+8,+11,+12,+20,+20,t(4;13)(q13;q34),del(6)(q22).

SUMMARY

There were a total of 22 patients who had t(11;22) or 22q− (one patient) in either the direct and/or tissue culture samples. The number of patients with numerical and structural abnormalities in 50% or more of the cells are plotted in Figure 12. In addition to chromosomes #11 and #22, chromosomes #1, #2, and #6 were most frequently involved in structural abnormalities, and chromosomes #8 and #5 were most frequently involved in numerical abnormalities. Every chromosome pair was involved in structural and/or numerical abnormalities in at least one patient.

We were successful in obtaining satisfactory material for cytogenetic analyses of nearly 50% (11 of 24) of the cases examined by direct and/or short-term culture preparation of tumor tissue.

DISCUSSION

Detailed cytogenetic analyses were performed on 24 SRCT studied by direct or short-term culture (1–4 days); 22 established tumor cell lines were also examined. A t(11;22) translocation was observed in each of the 15 specimens from patients with Ewing's sarcoma of bone, in the material from both cases of peripheral neuroepithelioma, and in four of the specimens from patients with Askin's tumor. In the fifth patient with Askin's tumor, a deletion of 22q12 was detected. We obtained

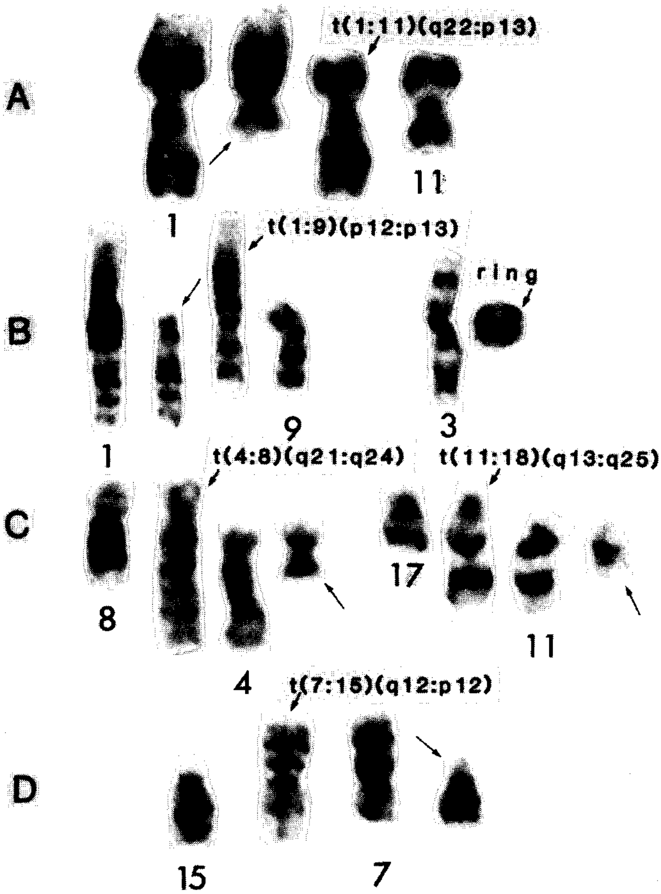


Figure 11 Four partial karyotypes from a patient 11-7 with ganglioneuroblastoma. (A) Pleural fluid: t(1;11)(q22;p13); (B–D) various structural abnormalities observed in stimulated peripheral blood preparation.

metaphase cells with 500–800 bands from the tumor from one patient with Ewing's sarcoma and these cells showed the breakpoints to be at band q23.2 in chromosome #11 and at band q11.2 in chromosome #22. In accordance with the Paris Conference Nomenclature Standardization [3], we designated the resulting translocation t(11;22)(q24;q12). It is of interest that the tumor from the patient with soft Ewing's sarcoma, which had evidence of vascular origin by electron microscopy, did not demonstrate evidence of t(11;22), raising the possibility that this tumor may represent a very primitive angiosarcoma that is unrelated in origin to classical Ewing's sarcoma of bone.

Each of the four direct cytogenetic examinations of tumor specimens from patients with Ewing's sarcoma contained t(11;22). Breakpoints on chromosome #11 were observed at q24 in three cases and at a higher breakpoint, t(11;22)(q21;q12), in the fourth case. All 13 cell lines established from Ewing's sarcoma also had t(11;22): 10 had t(11;22)(q24;q12), two had a complex translocation involving a third chromosome (chromosome #17 or #18), and one had a different breakpoint on chromosome #11, t(11;22)(q21;q12). The most complex translocation involving chromosomes #11 and #22, t(2;11;22;21), was observed in the Askin's cell line SKNMC; other abnormalities in this line included +8, −10,t(1;3)(q22;q27), and t(1;4)(p36;p13). This line had been studied previously by Biedler and Spengler nearly nine years ago [6]. At that time they reported modal numbers of 46 and 47,

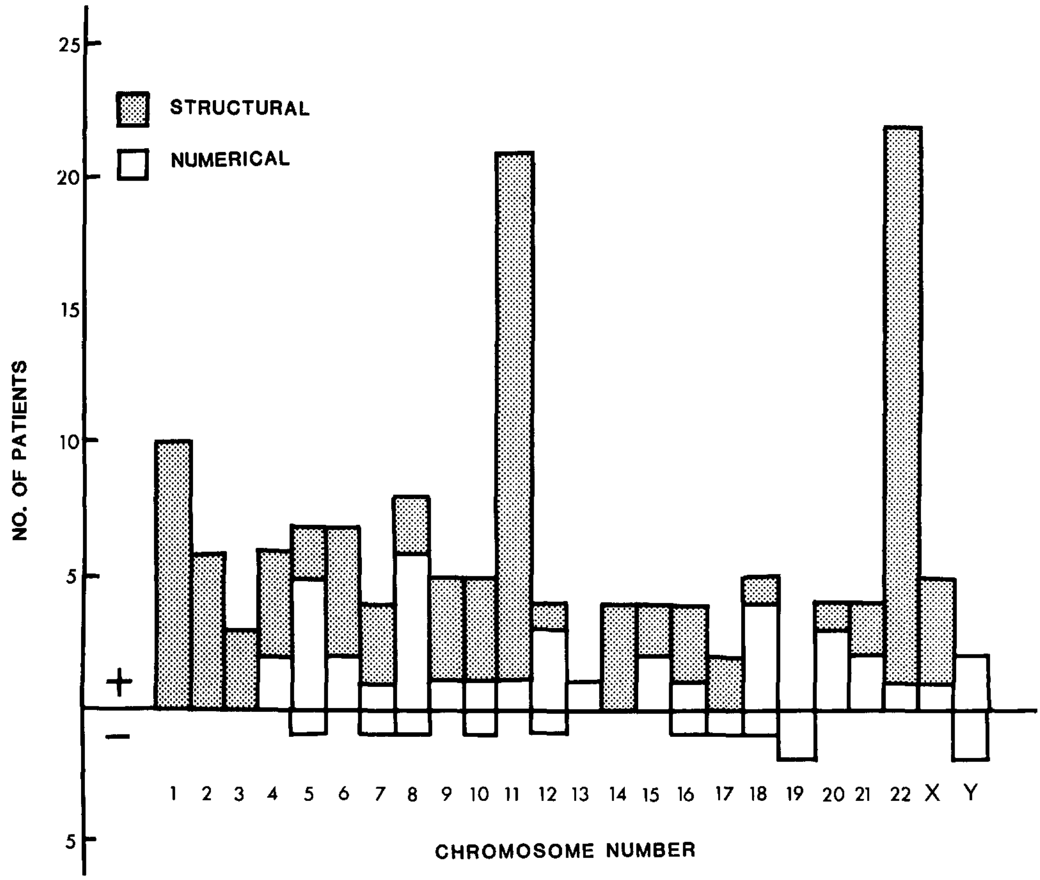


Figure 12 Incidence of numerical and structural chromosomal abnormalities in 22 patients with t(11;22).

numerical abnormalities including +6, −10, and −15, and structural abnormalities, such as inv(11)(p15q23), inv(16)(p11q24)?,t(2;3)(q11;q26.2), t(5;10)(q11;q21), t(5;10()q13;q21), and t(21;22)(p11;q11). The only similarities between their findings and ours are the −10 and the t(21;22) marker. The observation that chromosome numbers close to the diploid range are found in the direct examination of tumor specimens, while numerous numerical and structural changes with hyperdiploid or tetraploid chromosome numbers are present in the established cell lines, strongly suggests that the cell lines are karyotypically unstable. Our finding of extensive changes in SKNMC since its previous description is compatible with this interpretation.

In reviewing the structural and numerical chromosomal abnormalities in 22 patients with a t(11;22) marker we found that all chromosomes, including the sex chromosomes, were involved in at least one case (Fig. 12). Chromosome #1 was most frequently involved in structural abnormalities, followed by chromosomes #2, #4, #6, #9, #10, #14, and X. Chromosome #8 was most frequently involved in numerical abnormalities, followed by #5, #18, #12, and #20.

Turc-Carel et al. [4] reported the results of their cytogenetic studies in five Ewing's cell lines. They observed partial trisomies of chromosomes #1 (1q21-q31) and #8 (8q24.1-q24.2) in three of five and five of five of the lines, respectively. Douglass et al. [7] noted nonrandom deletions of chromosomes #1 and #3 in their series of Ewing's sarcoma patients. Although chromosome #1 was very frequently involved in structural abnormalities in the present series, no specific site could be identified as being nonrandom.

Neither homogeneously staining regions (HSR) nor double minutes (DM) were noted in any of the cytogenetic analyses of our samples either by direct study or by short- or long-term cultures. In contrast, Turc-Carel et al. [4] found these abnormalities in all five lines studied; however, the frequency of cells displaying either of these abnormalities varied greatly, both within and among these cell lines. HSR and DM occur more frequently in long-term than in short-term culture, and usually in cell lines established from patients who have received treatment [8]. The preponderance of our patients were examined prior to treatment, perhaps explaining the absence of these markers in our direct preparations. Further, because most of the cell lines we examined were established prior to therapy, and because most of the lines were examined in low passage numbers, it is possible that our cell lines differ significantly from those examined by previous investigators.

The one case (13-2) of soft tissue Ewing's sarcoma we examined had a translocation involving chromosomes #6 and #12; a translocation involving the same chromosomes but rearranged at different sites, t(6;12)(q14;p13), was seen in one of the patients with classic Ewing's sarcoma of bone. This finding and the absence of t(11;22) raises the possibility that soft tissue Ewing's sarcoma is a tumor significantly different in origin and pathogenesis from Ewing's sarcoma of the bone. More such cases should be studied to determine the significance and incidence of this abnormality in soft tissue Ewing's sarcoma.

Two cases of rhabdomyosarcoma were studied, one by direct analysis of tumor aspirated from the bone marrow (4-2) and one by long-term culture (TC-131T). Tumor from one patient (4-2) had two cell populations, one normal diploid, and one near-tetraploid (84–88 chromosomes), which showed t(2;11)(q27;q13),i(10q), and 14q+; however, no more details concerning other abnormalities could be determined due to the poor quality of the near-tetraploid line. The cell line TC-131T showed hyperdiploidy, del(6)(q22), and t(4;13)(q13;q34). Not only was the t(11;22) not seen, but the two tumors did not have any chromosomal abnormalities in common. The single case of ganglioneuroblastoma had multiple numerical and structural changes but we were unable to detect clonal formation in either pleural fluid or stimulated peripheral blood; one cell from each sample showed involvement of chromosome #11.

It is noteworthy that the protooncogene c-*sis* is located distal to 22q11 [9]. DNA hybridization studies in peripheral neuroepithelioma and Ewing's sarcoma have shown that c-*sis* is translocated to chromosome #11 in both diseases [10, 11]; however, both our results [10] and those of Bechet et al. [12] have shown the c-*sis* gene to be neither activated nor rearranged in Ewing's sarcoma. Recently, a new oncogene, v-*ets*, a second oncogene found in the E26 strain of avian myeloblastosis virus has been identified 3′ to v-*myb* [13, 14]. Leprince et al. [14] recently reported that v-*ets* has a cellular counterpart (c-*ets*) in chicken and human DNA. In humans it is located at 11q23-24; however, Southern blot analysis of the DNA from five different Ewing's sarcoma cell lines by de Taisne et al. [15] has thus far failed to show detectable rearrangement of v-*ets*. The involvement of either c-*sis* or c-*ets* in the malignant transformation of t(11;22)-related neoplasia remains to be studied.

A constitutional rearrangement, t(11;22)(q23;q11), observed in 32 unrelated families [16], involves breakpoints very similar to those seen in Ewing's sarcoma, peripheral neuroepithelioma, and Askin's tumor [5, 17–19]. However, there is no history of increased malignancies in these families with this constitutional rearrangement. The identification of chromosomal rearrangement at specific sites in both Ewing's sarcoma and the peripheral neuroectodermal tumors, and the possibility that known oncogenes are involved in their pathogenesis, are important considerations in our pursuit of the mechanisms that regulate the development of these tumors.

Chromosome region 11q22-25 was observed by Abe and Sandberg [20] to be significantly involved in chromosomal abnormalities in the monocytoid type of childhood acute leukemia, especially in infants; however, involvement of segment 11q11-21 was not noted in these patients. They suggested that there may be a relationship between the origin(s) or mechanism(s) of acute monocytic leukemia of childhood and infant acute leukemia. The involvement of chromosome bands 11q22-25 in these malignancies, as well as in the tumors described in this report, may indicate a correlation between events during pregnancy and development of neoplasms characterized by abnormalities of the 11q22-25 region, as suggested by Abe and Sandberg. Chromosome #22 is also involved in other types of neoplasia, most notably in CML and Burkitt's lymphoma.

In conclusion, in our study of small, round, blue-cell tumors we have found t(11;22) in tumors with evidence of neural origin, peripheral neuroepithelioma, and the Askin's tumor that is indistinguishable from the translocation seen in Ewing's sarcoma, a tumor of unknown histogenesis. The possibility that these translocations, in fact, are functionally identical may indicate that there is a similar tissue of origin for these two distinct histopathologic entities. Although our limited number of other SRCT did not demonstrate this translocation, further studies may reveal unsuspected similarities among these tumors.

REFERENCES

1. Triche TT (1982): Round cell tumors in childhood: The application of newer techniques to the differential diagnosis. In: Perspectives in Pediatric Pathology, Vol. 7, H Rosenberg, J Bernstein, eds. Masson, New York, pp. 279–322.
2. Seabright M (1971): A rapid banding technique for human chromosomes. Lancet ii:971–972.
3. ISCN (1978): An International System for Human Cytogenetic Nomenclature (1978): Birth Defects: Original Article Series, Vol. XIV, No. 8. The National Foundation, New York, pp. 309–404.
4. Turc-Carel C, Philip I, Berger M, Philip T, Lenoir GM (1984): Chromosome study of Ewing's sarcoma (ES) cell lines. Consistency of a reciprocal translocation t(11;22)(q24;q12). Cancer Genet Cytogenet 12:1–19.
5. Whang-Peng J, Triche TJ, Knutsen T, Miser J, Douglass EC, Israel MA (1984): Chromosome translocation in peripheral neuroepithelioma. N Engl J Med 311:584–585.
6. Biedler JL, Spengler BA (1976): A novel chromosome abnormality in human neuroblastoma and antifolate-resistant Chinese hamster cell lines in culture. J Natl Cancer Inst 57:683–695.
7. Douglass E, Green A, Hayes A, Brodeur G, Thompson E (1983): Deletion of chromosomes 1 and 3 in Ewing's sarcoma. Am Assoc Cancer Res 24(abstr):11.
8. Whang-Peng J, Bunn PA, Kao-Shan CS, Lee EC, Carney DN, Gazdar A, Minna JD (1982): A nonrandom chromosomal abnormality, del 3p(14-23), in human small cell lung cancer (SCLC). Cancer Genet Cytogenet 6:119–143.
9. Favera RD, Gallo RC, Giallongo A, Croce CM (1982): Chromosomal localization of the human homolog (c-*sis*) of the simian sarcoma virus *onc* gene. Science 218:686–688.
10. Thiele CJ, Whang-Peng J, Kao-Shan CS, Gelmann EP, Miser J, Israel MA (1985): Tranlocation of c-*sis* oncogene in peripheral neuroepithelioma. (submitted)
11. van Kessel AG, Turc-Carel C, de Klein A, Grosveld G, Lenoir G, Bootsma D. (1985): Translocation of oncogene c-*sis* from chromosome 22 to chromosome 11 in a Ewing sarcoma-derived cell line. Molec Cell Biol 5:427–479.

12. Bechet JM, Bornkamm G, Freese U-K, Lenoir GM (1983): The c-*sis* oncogene is not activated in Ewing's sarcoma. N Engl J Med 310:393.
13. Roussel M, Saule S, Lagrou C, Rommens C, Beug H, Graf T, Stehelin D (1979): Three new types of viral oncogene of cellular origin specific for haematopoietic cell transformation. Nature 281:452–455.
14. Leprince D, Gegonne A, Coll J, de Taisne C, Schneeberger A, Lagrou C, Stehelin D (1983): A putative second cell-derived oncogene of the avian leukaemia retorvirus E26. Nature 306:395–397.
15. de Taisne C, Gegonne A, Stehelin D, Bernheim A, Berger R (1984:) Chromosomal localization of the human proto-oncogene c-*ets*. Nature 310:581–583.
16. Zachai EH, Emanuel BS (1980): Site-specific reciprocal translocation, t(11;22)(q23;q11), in several unrelated families with 3:1 meiotic disjunction. Am J Med Genet 7:507–521.
17. Aurias A, Rimbaut C, Buffe D, Zucker J-M, Mazabraud A (1984): Translocation involving chromosome 22 in Ewing's sarcoma. Cancer Genet Cytogenet 12:21–25.
18. de Chadarevian J-P, Vekemans M, Seemayer TA (1984): Reciprocal translocation in small-cell sarcomas. N Engl J Med 311:1702–1703.
19. Whang-Peng J, Triche TJ, Knutsen T, Miser J, Israel MA (1984): Reciprocal translocation in small-cell sarcoma. N Engl J Med 311:1703.
20. Abe R, Sandberg AA (1984): Significance of abnormalities involving chromosomal segment 11q22-25 in acute leukemia. Cancer Genet Cytogenet 13:121–127.

APPENDIX

Ewing's Sarcoma Patients

3-13: 13-year-old male with Ewing's sarcoma of the right thenar eminence with metastasis to the thoracic spine. He was treated with intensive chemotherapy and local radiation followed by consolidation with total body irradiation and autologous bone marrow transplantation. He is now free of disease, 11 months after diagnosis.

2-16: 15-year-old male with Ewing's sarcoma of the jaw with metastases to the bone marrow. He was treated with intensive chemotherapy and local irradiation initially, then consolidation with total body irradiation and autologous bone marrow transplantation. He relapsed 15 months after diagnosis and died 19 months after diagnosis.

17-11: 5-year-old male with Ewing's sarcoma localized to the femur (without metastases). He was treated with intensive chemotherapy and local irradiation and is now free of disease, 6 months after diagnosis.

2-19: 10-year-old male with Ewing's sarcoma localized to the tibia (without metastases). He was treated with chemotherapy and local irradiation and is now free of disease, 6 months after diagnosis.

10-20: 16-year-old male with Ewing's sarcoma of the zygomatic bone without metastases. He began therapy with intensive chemotherapy but refused further treatment after two courses.

19-4: 23-year-old male with the vascular Ewing's variant (not classical Ewing's sarcoma) of the pelvis with pulmonary metastases. He was treated with intensive chemotherapy, local irradiation, thoracotomy, and intraoperative irradiation to the primary tumor mass, followed by total body irradiation and autologous bone marrow transplantation. He is alive with the disease, 10 months from diagnosis.

5-23: 15-year-old male with Ewing's sarcoma localized to the femur (without metastases). He was treated with intensive chemotherapy and local irradiation and is now free of disease, 6 months after diagnosis.

10-2: 37-year-old male who presented with a large pelvic tumor with involvement of the iliac bone and extension into the soft tissue by a large tumor mass. Extensive bilateral pulmonary metastases and bony metastases were present at diagnosis. Histologically, the primary tumor was consistent with Ewing's sarcoma and, of great interest, the histology was identical to the histology of his brother's bone tumor that developed 5 years previously. Further, his son developed and died of acute lymphocytic leukemia. He was treated with chemotherapy and irradiation to the primary and residual metastatic deposits. In spite of this therapy he only achieved a partial response and 18 months following the diagnosis he had progression of this disease that resulted in death.

Ewing's Sarcoma—Tissue Culture Lines

TC 71: This cell line was derived from a 22-year-old male with metastatic Ewing's sarcoma that arose in the humerus. The tumor was PAS-positive. The cell line was derived in 1981 from a biopsy of recurrent tumor at the primary site.

TC 106: This cell line was derived from a 19-year-old male with widespread metastatic Ewing's sarcoma that arose in the pelvis with metastases to bone, bone marrow, and lung. The tumor demonstrated intracytoplasmic glycogen on both light and electron microscopy. The cell line was derived in 1982 from a scalp mass prior to treatment.

A4573: This cell line was derived from a 17-year-old female with metastatic Ewing's sarcoma that originated in the clavicle and was metastatic to bone and lungs. The primary tumor consisted of round cells with scant cytoplasm that were PAS-positive. The cell line was derived in 1976 from pleural fluid following therapy and relapse.

5838: This cell line was derived from a 27-year-old male with metastatic Ewing's sarcoma that arose in the radius and was metastatic to lungs and pleura. The primary tumor consisted of round cells with hyperchromatic nuclei and clumped chromatin. Intracellular glycogen was confirmed by the PAS stain. The cell line was derived in 1974 from pleural fluid following therapy and relapse.

6647: This cell line was derived from a 14-year-old female with metastatic Ewing's sarcoma that arose in the proximal tibia. The tumor appeared as small round cells by light microscopy, which demonstrated intracytoplasmic glycogen. The cell line was derived in 1974 from pleural fluid following therapy and relapse.

A9423: This cell line was derived from a 13-year-old female with metastatic Ewing's sarcoma that arose in the pubic bone with metastases to bone and lung. The tumor was composed of small round cells with a fine chromatin pattern, inconspicuous nucleoli, and cytoplasmic glycogen. The cell line was derived in 1977 from a metastatic nodule following treatment and relapse.

N1001: This cell line was derived from an 11-year-old female with widespread metastatic Ewing's sarcoma that arose in the ilium with metastases to bone and bone marrow. Light and electron microscopy demonstrated small round cells with hyperchromatic nuclei, small glycogen deposits, and primitive cell–cell attachments consistent with Ewing's sarcoma. The cell line was derived in 1983 from a bone marrow aspiration following therapy and relapse.

N1002: This cell line was derived from a 25-year-old male with Ewing's sarcoma that arose in the rib with metastases to the pleura, lung, mediastinum, heart, and retroperitoneum, and subsequently, bone marrow. The tumor was composed of small round cells with round to oval nuclei and indistinct cytoplasm with abundant glycogen. The cell line was derived in 1983 from a bone marrow aspiration following therapy and relapse.

N1003: This cell line was derived from an 18-year-old male with metastatic Ewing's sarcoma that arose in the proximal tibia with metastases to the bone, bone marrow, and lung. The tumor was composed of small cells with round nuclei with intracytoplasmic glycogen. The cell line was derived in 1983 from a bone marrow aspiration following therapy and relapse.

N1007: This cell line was derived from a 19-year-old male with metastatic Ewing's sarcoma that arose in the humerus with metastases to the bone and bone marrow. The tumor was composed of small round cells with intracytoplasmic glycogen. The cell line was derived in 1984 from a bone marrow aspiration following treatment and relapse.

Askin's Tumor

19-19: 22-year-old male with a chest wall Askin's tumor with widespread metastases to bone and bone marrow. He has been treated with intensive chemotherapy and local radiation and is alive without evidence of disease, 6 months after diagnosis.

19-7: 15-year-old female with a chest wall Askin's tumor with widespread metastases to the bone and bone marrow. She was treated with intensive chemotherapy, local irradiation, total body irradiation, and autologous bone marrow transplantation. She relapsed 8 months after diagnosis and is alive and responding to chemotherapy, 1 year after diagnosis.

20-8: 31-year-old female with Askin's tumor localized to the right paraspinal area at T2. She was treated with intensive chemotherapy, local irradiation, total body irradiation, and autologous bone marrow transplantation. She relapsed 7 months after diagnosis and died 1 year after diagnosis.

CHP100: This tumor cell line was established in 1972 at the Children's Hospital of Philadelphia from a child with a mediastinal primary neuroectodermal tumor with paraspinal extension and bone metastases without an adrenal primary.

TC SKNMC: This tumor cell line was established from a patient with neuroectodermal tumor of the chest wall at Memorial Sloan Kettering Cancer Center (reported in 1973).

Peripheral Neuroepithelioma

10-15: 5-year-old male with a peripheral neuroepithelioma of the thigh with metastasis to lymph node. He was treated with intensive chemotheray and local irradiation. He is alive with no evidence of disease, 9 months after initial diagnosis.

11-15: 17-year-old female with peripheral neuroepithelioma of left ilium and adjacent soft tissue. No catecholamines were detected. Despite intensive chemotherapy, the patient developed widespread metastases and died within 1 year. The cell line was established from a rebiopsy of the primary tumor mass prior to treatment in 1979.

20-3: 14-year-old male with peripheral neuroepithelioma of soft tissue posterior and medial to femur with widespread disease involving bone, bone marrow, lung, and epidural space. There was no evidence of an adrenal mass, catecholamine levels were normal, and epinephrine levels were elevated.

Rhabdomyosarcoma

4-8: 12-year-old female with extremity alveolar rhabdomyosarcoma with nodal metastases. She was treated with intensive chemotherapy, local irradiation, total body irradiation, and autologous bone marrow transplantation. She relapsed 12 months after diagnosis and died 14 months after diagnosis.

4-2: 9-year-old female with alveolar rhabdomyosarcoma metastatic to lymph node, bone and bone marrow with a primary in the thigh. She was treated with intensive chemotherapy and local radiation therapy. She is now alive with no evidence of disease, 6 months after diagnosis.

TC131: This tumor cell line was established from a 19-year-old male with a left chest wall (soft tissue) tumor. Electron microscopy showed cytofilaments typical of primitive rhabdomyosarcoma. The tissue culture was established in August 1983 and cloned.

Soft Tissue Ewing's Sarcoma

10-2: 26-year-old male who presented with a large soft tissue mass arising in the thigh and with extensive pulmonary metastases. The light microscopic appearance of the primary tumor and pulmonary metastases were indistinguishable from Ewing's sarcoma of bone. However, ultrastructural features demonstrated evidence of vascular channel formation by tumor cells and cell–cell attachments suggestive of endothelial cells. He was treated with intensive chemotherapy, local irradiation, thoracotomy, total body irradiation, and autologous bone marrow transplantation. He relapsed 8 months after the diagnosis was made and is now responding to further chemotherapy.

Ganglioneuroblastoma

11-7: 19-year-old male with ganglioneuroblastoma that arose in the adrenal gland and was metastatic to bone and bone marrow. He was treated with intensive chemotherapy and local irradiation; however, in spite of this treatment, the tumor recurred and he subsequently died after attempts at retrieval chemotherapy. The cytogenetic evaluation was undertaken at the time of relapse.